普鲁士蓝染色试剂盒（中性红法）

货号： G1420
规格： 2×50mL/2×100mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称		2×50mL	2×100mL	保存
试剂（A）：Perls 染色工作液	A1：Perls 染色液 A	25mL	50mL	室温，避光
	A2：Perls 染色液 B	25mL	50mL	室温
临用前，取 A1、A2 等量混合，即为 Perls stain，不宜提前配制。				
试剂(B): 中性红染色液		50mL	100mL	室温，避光

产品介绍：

含铁血黄素（Hemosiderin）是一种血红蛋白源性色素，为金黄色或棕黄色颗粒，因其含铁、金黄色，故称为含铁血黄素。当红细胞被巨噬细胞吞噬后，在溶酶体酶的作用下，血红蛋白被分解为不含铁的橙色血质和含铁的含铁血黄素。

Perls 普鲁士蓝反应（Prussian blue reaction）又称为含铁血黄素染色，即经过亚铁氰化钾和稀酸处理后可以产生蓝色，常见于吞噬细胞内会间质内，主要显示三价铁盐。Perls 普鲁士蓝是非常经典的组织化学反应，是显示组织内三价铁的一种敏感、传统优良的方法，其染色原理为：亚铁氰化钾溶液使三价铁离子从蛋白质中被稀盐酸分离出来，三价铁与亚铁氰化钾反应，生成一种不溶解的蓝色化合物即三价铁的亚铁氰化物普鲁士蓝，所以该反应被称为普鲁士蓝反应。三价铁的亚铁氰化物是一种很稳定的化合物，在反应后可用红色染色剂进行复染，如核固红、伊红、中性红等。

Perls stain 常用于显示局部组织内各种出血性病变，常见于吞噬细胞内。在判断含铁血黄素沉积时，用 Perls 反应可以得到证实，该染色方法可以很好的区分含铁血黄素和其他色素。该染色液稳定性好、可以长期保存、不易产生沉淀、应用范围广、可以进行复染。

自备材料：

10%的中性福尔马林固定液、系列乙醇 、蒸馏水 、4%的多聚甲醛

操作步骤：(*仅供参考*)

（一）石蜡切片染色

1、 组织固定于 10%中性福尔马林，常规脱水包埋。
2、 切片厚度 4um，常规脱蜡至水。蒸馏水洗 1min.。
3、 切片入 Perls 染色工作液（见注意事项 4），浸染 15～30min ，蒸馏水充分冲洗 2～5min 。
4、 入中性红染色液，淡染细胞核 15～30s，自来水冲洗 1～5s。
5、 常规脱水透明，中性树胶封固。

（二）冰冻切片染色

1、 无需脱蜡，直接迅速用蒸馏水冲洗 2～3min。
2、 染色、脱蜡、透明、封固步骤同石蜡切片的染色步骤，时间可以相应缩短。

（三）细胞染色

1、 4%多聚甲醛固定 10～20min。
2、 蒸馏水冲洗 2 次，每次 2min。
3、 染色、脱蜡、透明、封固步骤同石蜡切片的染色步骤，时间可以相应缩短。

染色结果：

含铁血黄素或三价铁	蓝色
细胞核、其他组织	红色

阴性对照（*可选*）

取相同连续切片脱蜡至水。置于 5%的草酸中，孵育 2～6h 后，经 Perls 染色后结果为阴性。

注意事项：

1、 切片脱蜡应尽量干净。
2、 组织固定常采用 10%的中性福尔马林，经普通福尔马林长期固定后，组织会有损伤。避免使用酸性固定剂，铬酸盐处理也会妨碍铁的保存。
3、 整个操作过程中容器要干净，避免使用金属铁制品，洗切片和容器时以蒸馏水为宜，因普通水内含铁质。
4、 Perls stain 染色时，应根据样品情况调整着色时间。
5、 所有切片都应使用同一个阳性对照切片，选择适合的对照非常重要。尸检肺组织是一个很好的对照，包含相当数量的铁阳性巨噬细胞(心衰细胞)。
6、系列乙醇应经常更换新液。
7、冰冻切片和细胞的染色，应根据具体情况摸索实验条件。
8、为了您的健康和安全，请穿实验服并戴一次性手套操作。

Prussian Blue Iron Stain Kit(With Neutral Red)

Cat: G1420
Size: 2×50mL/2×100mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		2×50mL	2×100mL	Storage
Reagent(A): Perls Stain	A1：Perls Stain A	25mL	50mL	RT, avoid light
	A2：Perls Stain B	25mL	50mL	RT
Before use, mix equal parts of A1 and A2 to form Perls Stain. It is not suitable to prepare in advance.				
Reagent(B): Neutral Red Solution		50mL	100mL	RT, avoid light

Introduction

Hemosiderin is a hemoglobin derived pigment, which is golden yellow or brownish yellow particles. Because it contains iron and golden yellow, it is called hemosiderin. When the red blood cells are engulfed by macrophages, under the action of lysosomal enzymes, hemoglobin is broken down into iron free orange blood and iron-containing hemosiderin.

Perls Prussian blue reaction, also known as hemosiderin staining, can produce blue after being treated with potassium ferrocyanide and dilute acid, which is common in the interstitium of phagocytes, mainly showing ferric iron salts. Perls Prussian blue reaction is a very classical histochemical reaction, which is a sensitive and traditional excellent method to display the ferric iron in tissues. Its dyeing principle is: potassium ferrocyanide solution separates the ferric iron from the protein by dilute hydrochloric acid, and the ferric iron reacts with potassium ferrocyanide to form an insoluble blue compound named Prussian blue. Ferrocyanide of ferric iron is a very stable compound, which can be re-dyed with red dye after reaction, such as nuclear fast red, eosin, neutral red, etc.

Perls Stain is often used to display various hemorrhagic lesions in local tissues, and it is common in phagocytes. Perls reaction can be used to determine the deposition of hemosiderin, and this staining method can distinguish hemosiderin from other pigments. The dyeing solution has good stability, can be preserved for a long time, is not easy to produce precipitation, has a wide range of applications, and can be used for re-dyeing.

Self Provided Materials

10% netural formalin fixative, Series of ethanol, Distilled water, 4% paraformaldehyde

Protocol(*for reference only*)

（一）For paraffin section staining

1. Fix the tissue in 10% netural formalin fixative, dehydrate and embed.
2. Cut section in 4um thick and dewax to distilled water.Rinse in distilled water for 1min.
3. Soak the section in Perls Stain(***see note 4***) and stain for 15-30min. Rinse fully in distilled water for 2-5min.
4. Light staining the nucleus with Neutral Red Solution for 15-30s.Rinse in tap water for 1-5s.
5. Conventionally dehyarate and transparent, seal with resinene.

（二）For frozen section staining

1. Without dewaxing, rinse directly and quickly with distilled water for 2-3min.
2. Follow the other steps as paraffin sections.

（三）For cultured cell staining

1. Fix in 4% paraformaldehyde for 10-20min.
2. Rinse in tap water twice for each time 2min.
3. The steps of Staining, dehydration, transparency and sealing are the same as the steps of paraffin section. The time should be shortened accordingly.

Result

Hemosiderin or Ferric Iron	Blue
Nucleus and other Tissues	Red

Negative Control

Take the same adjacent section and dewax to water. After incubation in 5% oxalic acid for 2-6h, the

procedure is the same as above. The result should be negative.

Note

1. Section dewaxing should be as clean as possible.
2. 10% neutral formalin is often used for tissue fixation. After long-term fixation with common formalin, tissue will be damaged. Avoid the use of acid fixatives, chromate treatment will also hinder the preservation of iron.
3. During the whole operation process, the container should be clean and avoid the use of metal iron products. When washing sections and containers, distilled water is suitable, because ordinary water contains iron.
4. When dyeing with Perls Stain, the time should be adjusted according to the sample situation.
5. All sections should use the same positive control section, so it is very important to select the appropriate control. Autopsy lung tissue is a good control, containing a considerable number of iron positive macrophages (heart failure cells).
6. Series of ethanol should be replaced frequently.
7. For the staining of frozen section and cell, should explore the experimental conditions according to the specific conditions.
8. For your health and safety, please wear the experimental clothes and disposable gloves.